

Proposed Tertiary Olfactory Pathways in a Teleost, *Carassius auratus*

KEN OHNISHI

*Department of Physiology, Nara Medical University,
Kashihara, Nara 634, Japan*

ABSTRACT—Projection fields of the secondary olfactory terminal fields in the goldfish (*Carassius auratus*) were studied using retrograde axonal transport of horseradish peroxidase (HRP). The cell bodies within the ipsilateral lateral and posterior secondary olfactory terminal fields were labeled retrogradely after the injection of HRP into the medioposterior region of area dorsalis telencephali pars dorsalis (mpDd). The cell bodies within the hypothalamic secondary olfactory terminal field were labeled retrogradely after the injection of HRP into the posterior region of area dorsalis telencephali pars medialis (pDm). These anatomical results suggest that the mpDd and pDm in the goldfish probably form part of the tertiary olfactory projection fields.

INTRODUCTION

In the teleostean olfactory systems, the neural pathways of the secondary olfactory fibers have been studied by many investigators not only anatomically [1-10] but also electrophysiologically [11] for understanding of the evolution of the vertebrate central nervous systems. The projection fields of the fibers revealed in these studies are essentially very similar among different species. In the goldfish, *Carassius auratus*, Ichikawa [5], Bartheld *et al.* [1] and Levine and Dethier [7] have pointed out that the secondary olfactory fibers project bilaterally to three main telencephalic regions: (1) ventral area surrounding the dorsal aspects of the medial olfactory tract (medial terminal field of Ichikawa [5]); (2) medioventral region of area dorsalis telencephali pars lateralis (lateral terminal field of Ichikawa [5]); (3) posteroventral region of area dorsalis telencephali pars lateralis (posterior terminal field of Ichikawa [5]) and one diencephalic region: nucleus posterior tuberis, NPT, (hypothalamic terminal field of Bartheld *et al.* [1]). However, projections of the secondary olfactory terminal fields in the telencephalic hemispheres of teleosts have not been

investigated as yet except for Murakami *et al.* [8]. To examine whether the higher olfactory terminal fields exist or not in teleosts, axon terminal fields of the neurons within lateral, posterior and hypothalamic terminal field were examined using retrograde labeling with horseradish peroxidase (HRP).

MATERIALS AND METHODS

Sixty goldfish (*Carassius auratus*), 10 to 13 cm in length, obtained from a commercial dealer were used. The animals were anesthetized by putting them in a small tank containing 0.1% tricaine methanesulfonate (MS 222) and then positioned in the stereotaxic apparatus slightly modified from that of Peter and Gill [12]. During surgery, the animals were respired by circulating aerated tap water containing 0.05% MS 222 through the gill. The dorsal part of the skull was removed with a dental drill and the brain was exposed under a dissecting microscope. HRP injection into the surface layer of the telencephalon was performed by the insect pin method. An insect pin (No. 000) coated with a small amount of HRP paste was manually inserted into the desired site at the surface layer of the telencephalon [13]. After survival times of 3 days in a water tank at 20-25°C, the animals were anesthetized with MS 222 and

then perfused through the conus arteriosus with 0.6% saline followed by a solution of 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed from the skull and postfixed in the same buffer solution containing 10% sucrose for 24 hr. Frontal 50 μm thick sections were cut on a cryostat while referring to the atlas of Peter and Gill [12]. The present terminology was after this reference [12]. The HRP reaction with tetramethylbenzidine was processed according to the procedure of Mesulam [14]. The sections mounted on the gelatin-coated slide were counterstained with 1% neutral red.

RESULTS AND DISCUSSION

HRP injections were performed in twelve regions of the telencephalic surface layer divided

into the anterior, anteromedial, medioposterior and posterior region of area dorsalis telencephali pars lateralis (Dl), dorsalis (Dd) and medialis (Dm), respectively. At the injection site, the diffusion of HRP was confined to a region of about 300 μm in diameter. After the injection into the mpDd, a large number of labeled cells were observed densely in the ipsilateral medioventral (Fig. 1 C and D) and posteroventral region of Dl (Fig. 1 E and F) and precommissural medioposterior region of area dorsalis telencephali pars centralis (Dc), (Fig. 1 B and C). The labeled cells in the posteroventral region of Dl were 5 to 7 μm in diameter (Fig. 3 a and b). A few labeled cells were also observed sparsely in the ipsilateral medioposterior region of Dl (Fig. 1 B), Dd (Fig. 1 C) and Dm (Fig. 1 B-D) and posterior region of Dm (Fig. 1 E and F). No labeled cells were observed in the

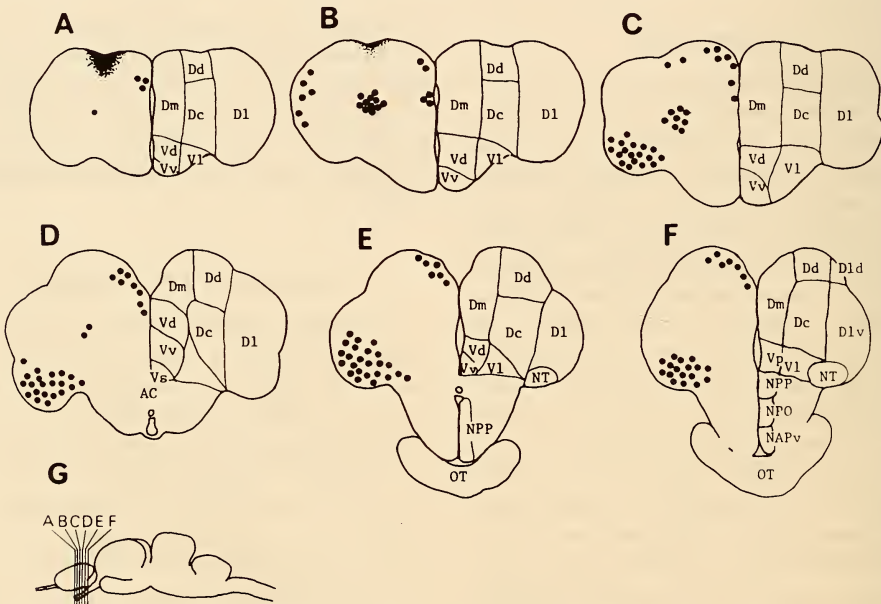


FIG. 1. A-F. The distribution of labeled cells in the telencephalon after injection of HRP into the mpDd. Blackened area and surrounding stippled area indicate the injection site and diffusion area of HRP, respectively. Each solid circle marks the location of several labeled cells. AC, anterior commissure; Dc, area dorsalis telencephali pars centralis; Dd, area dorsalis telencephali pars dorsalis; Dl, area dorsalis telencephali pars lateralis; Dld, area dorsalis telencephali pars lateralis dorsalis; Dlv, area dorsalis telencephali pars lateralis ventralis; Dm, area dorsalis telencephali pars medialis; NAPv, nucleus anterioris periventricularis; NPO, nucleus preopticus; NPP, nucleus preopticus periventricularis; NT, nucleus tenia; OT, optic tract; Vd, area ventralis telencephali pars dorsalis; Vl, area ventralis telencephali pars lateralis; Vp, area ventralis telencephali pars postcommissuralis; Vs, area ventralis telencephali pars supracommissuralis; Vv, area ventralis telencephali pars ventralis. G. Levels of A-F.

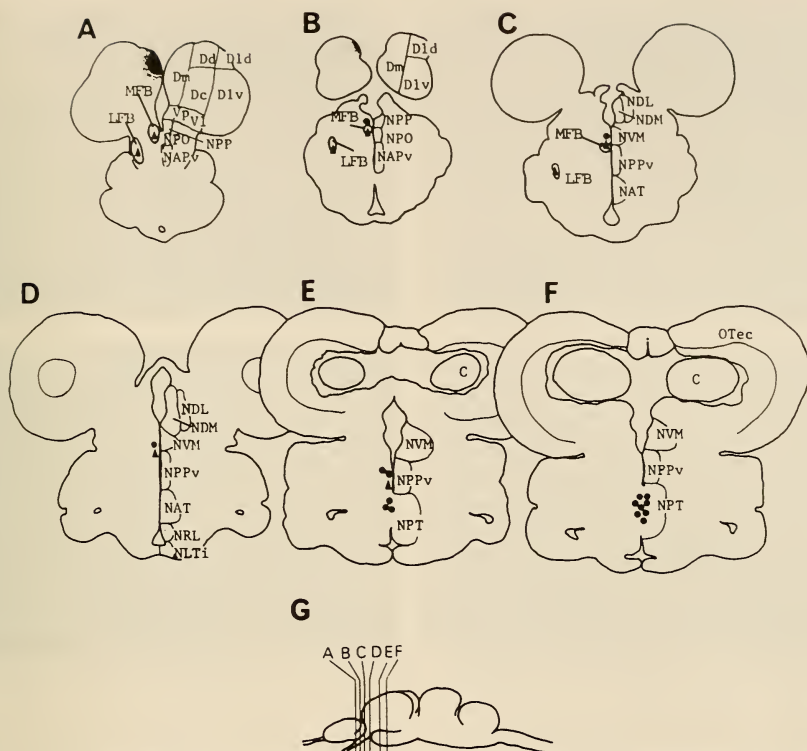


FIG. 2 A-F. The distribution of labeled cells in the telencephalon and diencephalon after injection of HRP into the pDm. Filled triangles indicate labeled axons. C, cerebellum; LFB, lateral forebrain bundle; MFB, medial forebrain bundle; NAT, nucleus anterior tuberis; NDL, nucleus dorsolateralis thalami; NDM, nucleus dorsomedialis thalami; NLTi, nucleus lateralis tuberis pars inferioris; NPPv, nucleus posterioris periventricularis ventralis; NRL, nucleus recessus lateralis; NVM, nucleus ventromedialis thalami; OTec, optic tectum. G. Levels of A-F. See Fig. 1 for further explanations.

medial terminal field. The medioventral and the posteroventral region of DI in the goldfish has been reported to receive the secondary olfactory fibers from the olfactory bulb [1, 5, 7]. Consequently, the labeled cells in the medioventral and posteroventral region of DI probably make a connection with the secondary olfactory fibers. After the injection into pDm, retrogradely labeled cells were mostly observed in the NPT (Fig. 2 E and F) and few labeled cells in the nucleus preopticus periventricularis (Fig. 2 B and E) and nucleus ventromedialis thalami (NVM, Fig. 2 C and D). The labeled cells in the NPT were very small and they were less than $3 \mu\text{m}$ in diameter (Fig. 3 c and d). Labeled axons were also observed in the medial forebrain bundle running ventral area of nucleus preopticus periventricularis and

NVM (Fig. 2 A-E) and some in the lateral forebrain bundle (Fig. 2 A and B). No labeled cells were observed in the medial, lateral or posterior terminal field. The NPT receives secondary olfactory fibers from the olfactory bulb in the goldfish [1, 7]. Thus, the labeled cells in the NPT probably connect with the secondary olfactory fibers. HRP injections into other regions scarcely led to retrograde labeling of cells in the medial, lateral, posterior or hypothalamic terminal field.

Mammalian central olfactory neural pathways can be divided into two main routes, direct projection from the secondary olfactory terminal field to the tertiary olfactory terminal field in the telencephalon [15-19] and another nondirect projection via diencephalon to the telencephalon [19-23]. However, in teleosts only direct projection is

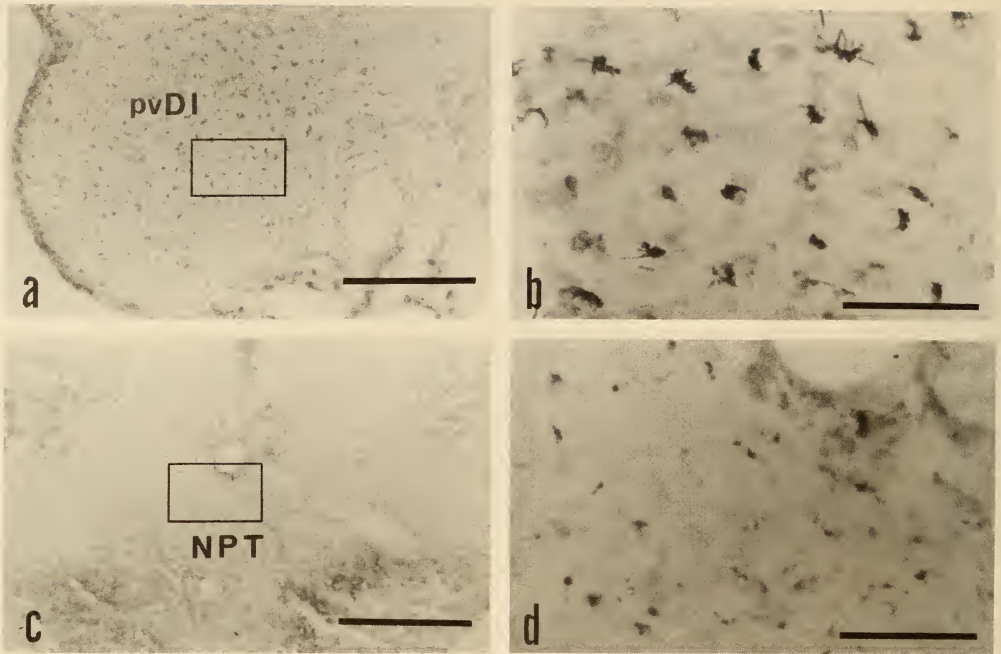


FIG. 3. a and b. Labeled cells in posteroventral region of area dorsalis telencephali pars lateralis (pvDI) after HRP injection into medioposterior region of area dorsalis telencephali pars dorsalis (mpDd). c and d. Labeled cells in nucleus posterior tuberis (NPT) after HRP injection into posterior region of area dorsalis telencephali pars medialis (pDm). The outlined area of a and c is shown at a higher magnification in b and d, respectively. Scale bar=300 μ m (a and c), 75 μ m (b and d).

known [8]. In the present study, nondirect projection via diencephalon was revealed for the first time in the teleostean olfactory system. This suggests that not only in mammals but also in teleosts two main olfactory routes exist. In the nondirect projection in the monkey, the hypothalamus receives the tertiary olfactory fibers from the secondary olfactory terminal field (piriform cortex) and projects to the telencephalic area (lateroposterior orbitofrontal cortex) [22]. Compared with this pathway, the route via the hypothalamus in the goldfish is more simple. The hypothalamus receives directly fibers from the olfactory bulb not via the secondary olfactory terminal field and projects to the telencephalic area. These results lead to the proposition that the central olfactory nervous systems become more complex with evolution. This view will be confirmed when olfactory projections of olfactory bulb-hypothalamus-telencephalon are detected in lower vertebrates such as amphibians and reptiles.

Murakami *et al.* [8] have detected in the rockfish, *Sebastes marmoratus*, retrogradely labeled cells in the ipsilateral posterior terminal field after HRP injection into ventral region of the Dm and concluded the region as the tertiary olfactory terminal field. The projection field of the posterior terminal field in the goldfish was the ipsilateral mpDd. This discrepancy may result from method differences. In present study, since HRP injections were strictly limited to the surface layer of the telencephalon, the projections to the inner parts of the telencephalon, i.e. ventral region of Dm, Dc, medial region of DI and area ventralis telencephali, were not examined. Thus, the possibility of the projections to the inner parts from the secondary olfactory terminal fields, especially the medial terminal field whose projection area are not detected, remain unclear. Furthermore, compared with projections of the secondary olfactory fibers, those of the tertiary olfactory fibers may differ greatly with the species. To prove

this hypothesis, investigations on the higher olfactory projections, i.e. projections of the secondary olfactory terminal fields in other teleostean species, are required.

REFERENCES

- 1 Bartheld, C. S., Meyer, D. L., Fiebig, E. and Ebbesson, S. O. E. (1984) Central connections of the olfactory bulb in the goldfish, *Carassius auratus*. *Cell Tissue Res.*, **238**: 475-487.
- 2 Bass, A. H. (1981) Olfactory bulb efferents in the channel catfish, *Ictalurus punctatus*. *J. Morphol.*, **169**: 91-111.
- 3 Ebbesson, S. O. E., Meyer, D. L. and Scheich, H. (1981) Connections of the olfactory bulb in the piranha (*Serrasalmus nattereri*). *Cell Tissue Res.*, **216**: 167-180.
- 4 Finger, T. E. (1975) The distribution of the olfactory tracts in the bullhead catfish, *Ictalurus nebulosus*. *J. Comp. Neurol.*, **161**: 125-142.
- 5 Ichikawa, M. (1975) The central projections of the olfactory tract in the goldfish, *Carassius auratus*. *J. Fac. Sci. Univ. Tokyo*, **13**: 257-263.
- 6 Ito, H. (1973) Normal and experimental studies on synaptic patterns in the carp telencephalon, with special reference to the secondary olfactory termination. *J. Hirnforsch.*, **14**: 237-253.
- 7 Levine, R. L. and Dethier, S. (1985) The connections between the olfactory bulb and the brain in the goldfish. *J. Comp. Neurol.*, **237**: 427-444.
- 8 Murakami, T., Morita, Y. and Ito, H. (1983) Extrinsic and intrinsic fiber connections of the telencephalon in a teleost, *Sebastiscus marmoratus*. *J. Comp. Neurol.*, **216**: 115-131.
- 9 Oka, Y. (1980) The origin of the centrifugal fibers to the olfactory bulb in the goldfish, *Carassius auratus*: An experimental study using the fluorescent dye primuline as a retrograde tracer. *Brain Res.*, **185**: 215-225.
- 10 Scalia, F. and Ebbesson, S. O. E. (1971) The central projections of the olfactory bulb in a teleost (*Gymnothorax funebris*). *Brain Behav. Evol.*, **4**: 376-399.
- 11 Fujita, I., Satou, M. and Ueda, K. (1984) A field-potential study of centripetal and centrifugal connections of the olfactory bulb in the carp, *Cyprinus carpio* (L.). *Brain Res.*, **321**: 33-44.
- 12 Peter, R. E. and Gill, V. E. (1975) A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carassius auratus*. *J. Comp. Neurol.*, **159**: 69-102.
- 13 Finger, T. E. (1978) Cerebellar afferents in teleost catfish (Ictaluridae). *J. Comp. Neurol.*, **181**: 173-182.
- 14 Mesulam, M.-M. (1978) Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J. Histochem. Cytochem.*, **26**: 106-177.
- 15 Allison, A. C. (1953) The morphology of the olfactory system in the vertebrates. *Biol. Rev.*, **8**: 195-244.
- 16 Gerfen, C. R. and Clavier, R. M. (1979) Neural inputs to the prefrontal agranular insular cortex in the rat: Horseradish peroxidase study. *Brain Res. Bull.*, **4**: 347-353.
- 17 Harberly, L. B. and Price, J. L. (1978) Association and commissural fiber systems of the olfactory cortex of the rat. I. Systems originating in the piriform cortex and adjacent areas. *J. Comp. Neurol.*, **178**: 711-740.
- 18 Krettek, J. E. and Price, J. L. (1977) Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J. Comp. Neurol.*, **172**: 687-722.
- 19 Potter, H. and Nauta, W. J. H. (1979) A note on the problem of olfactory associations of the orbitofrontal cortex in the monkey. *Neuroscience*, **4**: 361-367.
- 20 Giachetti, I. and MacLeod, P. (1977) Olfactory input to the thalamus: Evidence for a ventroposteromedial projection. *Brain Res.*, **125**: 166-169.
- 21 Powell, T. P. S., Cowan, W. M. and Raisman, G. (1965) The central olfactory connexions. *J. Anat.*, **99**: 791-813.
- 22 Tanabe, T., Yarita, H., Iino, M., Ooshima, Y. and Takagi, S. F. (1975) An olfactory projection area in orbitofrontal cortex of the monkey. *J. Neurophysiol.*, **38**: 1269-1283.
- 23 Yarita, H., Iino, M., Tanabe, T., Kogure, S. and Takagi, S. F. (1980) A transthalamic olfactory pathway to orbitofrontal cortex in the monkey. *J. Neurophysiol.*, **43**: 69-85.